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LATENT CLASS ANALYSIS OF HUMAN HERPESVIRUS 8 ASSAY PERFORMANCE AND INFECTION PREVALENCE IN SUB-SAHARAN AFRICA AND MALTA

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Human herpesvirus 8 (HHV-8) is thought to be highly prevalent in Mediterranean countries and sub-Saharan Africa, where it causes Kaposi's sarcoma in a small proportion of infected immunocompetent persons. However, the lack of serological tests with established accuracy has hindered our understanding of the prevalence, risk factors and natural history of HHV-8 infection. We tested 837 subjects from Congo, Botswana (mostly young adults) and Malta (elderly adults), using an immunofluorescence assay and 2 enzyme immunoassays (EIAs, to viral proteins K8.1 and orf65). Each assay found HHV-8 seroprevalence to be high (49–87%) in the African populations and generally lower (9–54%) in Malta. However, there was only modest agreement among tests regarding which subjects were seropositive (3-way κ, 0.05–0.34). We used latent class analysis to model this lack of agreement, estimating each test's sensitivity and specificity and each population's HHV-8 prevalence. Using this approach, the K8.1 EIA had consistently high sensitivity (91–100%) and specificity (92–100%) across populations, suggesting that it might be useful for epidemiological studies. Compared with the K8.1 EIA, both the immunofluorescence assay and the orf65 EIA had more variable sensitivity (80–100% and 58–87%, respectively) and more variable specificity (57–100% and 48–85%, respectively). HHV-8 prevalence was 7% among elderly Maltese adults. Prevalence was much higher (82%) in Congo, consistent with very high Kaposi's sarcoma incidence there. Prevalence was also high in Botswana (87% in Sans, an indigenous group, and 76% in Bantus), though Kaposi's sarcoma is not common, suggesting that additional co-factors besides HHV-8 are needed for development of Kaposi's sarcoma. *Int. J. Cancer* 88:1003–1008, 2000.
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Kaposi's sarcoma, a tumor most notably associated with the human immunodeficiency virus (HIV) epidemic, occurs in excess among apparently healthy individuals in certain well-defined geographical regions (Beral, 1991). One form of Kaposi's sarcoma (often termed "classic" Kaposi's sarcoma) arises in elderly adults of Mediterranean or Eastern European origin. Another form ("endemic" Kaposi's sarcoma) occurs in sub-Saharan Africa and can affect both children and adults.

Human herpesvirus 8 (HHV-8) is a necessary component cause of Kaposi's sarcoma. The virus is present in tumor tissues in all forms of Kaposi's sarcoma (Chang *et al.,* 1994; Huang *et al.,* 1995). However, among immunocompetent persons, only a small minority of those infected with HHV-8 develop Kaposi's sarcoma. Epidemiological studies of HHV-8 infection have relied on serological assays since viremia is detectable in few asymptomatically infected persons (LaDuca *et al.,* 1998; Whitby *et al.,* 1995). Using a variety of assays, HHV-8 antibodies are found in a large proportion of asymptomatic persons at increased risk for the tumor, *e.g.,* HIV-infected homosexual men (Simpson *et al.,* 1996; Gao *et al.,* 1996; Martin *et al.,* 1998); adults from Italy, Greece and various regions of sub-Saharan Africa (Olsen *et al.,* 1998; He *et al.,* 1998; Mayama *et al.,* 1998; Sitas *et al.,* 1999; Calabro *et al.,* 1998; Simpson *et al.,* 1996; Whitby *et al.,* 1998); and few persons at low risk, *e.g.,* blood donors from the United Kingdom, United States and Jamaica (Simpson *et al.,* 1996; Engels *et al.,* 1999).

Optimal methods for HHV-8 screening remain to be determined. Available serological tests are imperfectly sensitive since most do not identify all persons with Kaposi's sarcoma (Engels *et al.,* 2000; Rabkin *et al.,* 1998; Gao *et al.,* 1996; Simpson *et al.,* 1996). Furthermore, many Kaposi's sarcoma patients have much higher antibody levels than asymptomatically infected individuals (Engels *et al.,* 2000; Simpson *et al.,* 1996; Sitas *et al.,* 1999). Thus, published estimates of test sensitivity, which are based on samples from persons with Kaposi's sarcoma, may not be applicable for detection of asymptomatic infection. Finally, test specificity remains poorly estimated because it has been difficult to identify a representative population known to be uninfected (Engels *et al.,* 2000; Martin *et al.,* 2000).

The lack of serological tests with demonstrably high sensitivity and specificity prevents a more complete understanding of the prevalence, transmission and natural history of HHV-8 infection. For example, HHV-8 seroprevalence is high in Gambia (Ariyoshi *et al.,* 1998; Whitby *et al.,* 1999), but the incidence of Kaposi's sarcoma is low (Cook-Mozaffari *et al.,* 1998). This discordance raises the question of whether a co-factor, lacking in parts of western Africa, is needed for development of Kaposi's sarcoma. An alternative explanation is that serological assays have low specificity in this setting, due perhaps to the presence of other infections. Further complicating an examination of HHV-8 epidemiology has been the lack of agreement among HHV-8 tests regarding which individuals are infected (Rabkin *et al.,* 1998).

Latent class analysis is a valuable approach for modeling test results when disagreement is present and a "gold standard" reference test is lacking (Hui and Walter, 1980; Walter and Irwig, 1988). These methods have been applied to a variety of problems in the medical and social sciences (Sinclair *et al.,* 1998; Alvord *et al.,* 1988; Walter *et al.,* 1999; de Bock *et al.,* 1994). Latent class models assume that the observed test results derive from an unknown ("latent") prevalence of the condition of interest within a given population and from the unknown sensitivity and specificity of the various tests. Under certain conditions, these models provide estimates of prevalence and each test's sensitivity and specificity.

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In the present study, we assessed the performance of serological tests for HHV-8 infection and estimated the prevalence of HHV-8 infection in several populations at risk for Kaposi's sarcoma. Specifically, we used 3 different serological assays to categorize the infection status of subjects from Malta, the Democratic Republic of Congo (formerly Zaire) and Botswana. We then fitted latent class models to derive estimates for the sensitivity and specificity of each test and for HHV-8 prevalence within each population.

MATERIAL AND METHODS

Study subjects

Subjects from 3 sub-Saharan African populations were studied. We included 321 controls (155 male, 166 female; median age 29 years, range 6–78) recruited from a case-control study of Kaposi's sarcoma conducted in the Lake Kivu District of the Democratic Republic of Congo in 1984 (Kestens *et al.,* 1985). Also, we included 2 separate groups of adults participating in a 1987 community-based survey of HIV seroprevalence in Botswana (Ebbesen *et al.,* 1989). The first Botswana group comprised 155 semi-nomadic San people (81 male, 69 female, 5 unknown sex; age range 18–55), also known as Bushmen, living in the Kalahari desert. The second Botswana group comprised 180 Bantus (89 male, 91 female; age range 16–64 years) living in the small town of Ghanzi. Because of limited serum samples, we included only 161 Bantus (only grouped data on the Botswana subjects' sex and age were available from the original study, so the sex and age distributions of subjects were not known with certainty). Finally, for comparison, we included, from the Mediterranean island of Malta, 200 elderly adults from an outpatient clinic (100 male, 100 female; median age 78 years, range 71–100).

Laboratory assays

Serum or plasma specimens from these subjects (stored at –70°C) were tested for the presence of HHV-8 antibodies using an indirect immunofluorescence assay (IFA) and 2 enzyme immunoassays (EIAs) (Engels *et al.,* 2000). Specifically, the IFA detected antibodies to HHV-8 latency-associated nuclear antigen (LANA or LNA-1), using the latently HHV8-infected primary effusion lymphoma cell line BCP-1 (Boshoff *et al.,* 1998). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100 at room temperature. Serum or plasma samples were diluted 1:100 in phosphate-buffered saline (PBS) containing 3% fetal bovine serum (FBS) and incubated on slides at room temperature for 30 min. Slides were washed 3 times in PBS/FBS before 30 min incubation at room temperature with anti-human-IgG FITC conjugate diluted 1:35 (Roche, Indianapolis, IN) and counterstain/blocking solution diluted 1:15 (ViroStat, Portland, ME). Slides were washed once in PBS/FBS and 4 times in PBS and examined by UV microscopy, with subjects characterized as IFA-seropositive if stippled nuclear immunofluorescence was observed.

The 2 EIAs detected antibodies to either HHV-8 orf65 (a minor capsid protein) or K8.1 (a structural glycoprotein probably spanning the virus lipid envelope). These assays were developed in our laboratory, based on similar assays described by others (Raab *et al.,* 1998; Simpson *et al.,* 1996; Renwick *et al.,* 1998); the K8.1 EIA methods have been slightly modified from our previous description (Engels *et al.,* 2000). Using 0.05 M carbonate/bicarbonate buffer solution at pH 10.0, orf65 was diluted to a final concentration of 1.25 μg/ml and K8.1 to a final concentration of 1.00 μg/ml. Polysorp 96-well plates (Nalge Nunc, Naperville, IL) were coated by adding 100 μl of diluted protein to each well. Plates were covered and incubated overnight at 4°C and then washed 3 times with 350 μl/well of 10× wash solution (NEN Life Science Products, Boston, MA). Three hundred microliters of blocking solution (2.5% BSA, 2.5% normal goat serum and 0.005% Tween-20 in PBS, with 0.005% Triton X-100 added for the K8.1 EIA) were placed in each well. Plates were covered, incubated for 2.5 hr at 37°C and then washed 3 times with 350 μl of wash solution. Serum or plasma samples were diluted 1:20 in blocking solution, and 100 μl were added to each well. Plates were covered, incubated for 90 min at 37°C and then washed 5 times with 350 μl/well wash solution. One hundred microliters of goat anti-human–IgG alkaline phosphatase conjugate (Roche; 1:3,000 dilution in blocking buffer) were added to each well and the plates covered. After incubation at 37°C for 30 min, plates were again washed 5 times. One hundred microliters of substrate solution (pH 9.8, 10% diethanolamine, NaN_3, $MgCl\text{-}6H_2O$, 1 mg/ml para-nitrophenylphosphate) were added to each well and the plates covered. After 30 min at 37°C, 50 μl of 3 N NaOH stop solution were added to each well. Plates were read at 405 nm on an automated plate reader. Subjects were classified as EIA-seropositive if the optical density was above 0.50 (for the orf65 EIA) or 1.00 (for the new version of the K8.1 EIA, which was roughly equivalent to 1.50 in the prior version); these cut-offs were chosen to have high specificity, based on our prior work (Engels *et al.,* 2000).

Statistical analyses

For descriptive purposes, we used the κ statistic to characterize 3-way or pairwise agreement beyond chance among the tests (Siegel and Castellan, 1988; Sackett *et al.,* 1991). Following convention, κ values measure agreement as slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) or almost perfect (0.81–1.00).

We fitted latent class models to the test results (Hui and Walter, 1980; Walter and Irwig, 1988). For each of our 4 populations, these models provided estimates of the infection prevalence π and of the error rates of the 3 tests (false-positive errors α, which are 1 − specificity; false-negative errors β, which are 1 − sensitivity). Individuals within each group were classified into 1 of 8 (2 × 2 × 2) categories, based on their results (positive or negative) on each of the 3 tests. We derived expressions (using π, β and α) for the expected proportion of each sample falling in each category. These expressions depended on a "conditional independence" assumption, which posits that the probability of observing a combination of test results, given infection status, is the product of the separate marginal probabilities for each test result. For example, the proportion of subjects positive on all 3 tests was calculated as follows:

$$\begin{aligned} P\,(\text{positive on all tests}) &= p_{111} \\ &= P\,(\text{infected and true-positive on all tests}) \\ &+ P\,(\text{uninfected and false-positive on all tests}) \\ &= \pi\,(1-\beta_{\mathrm{orf65}})\,(1-\beta_{\mathrm{IFA}})\,(1-\beta_{\mathrm{K8.1}}) \\ &\qquad + (1-\pi)\,(\alpha_{\mathrm{orf65}})\,(\alpha_{\mathrm{IFA}})\,(\alpha_{\mathrm{K8.1}}) \end{aligned}$$

The proportion negative on all 3 tests was calculated as follows:

$$p_{000} = \pi\,(\beta_{\mathrm{orf65}})\,(\beta_{\mathrm{IFA}})\,(\beta_{\mathrm{K8.1}}) + (1-\pi)\,(1-\alpha_{\mathrm{orf65}})\,(1-\alpha_{\mathrm{IFA}})\,(1-\alpha_{\mathrm{K8.1}}).$$

Likewise, the proportion of subjects who were positive on the orf65 EIA and IFA but negative on the K8.1 EIA was calculated as follows:

$$p_{110} = \pi\,(1-\beta_{\mathrm{orf65}})\,(1-\beta_{\mathrm{IFA}})\,(\beta_{\mathrm{K8.1}}) + (1-\pi)\,(\alpha_{\mathrm{orf65}})\,(\alpha_{\mathrm{IFA}})\,(1-\alpha_{\mathrm{K8.1}})$$

Similar expressions were derived for each of the other 5 categories defined by combinations of test results.

Using these expressions and the observed proportions of individuals in each category, for each population we calculated maximum likelihood estimates for π, α (which yielded specificity estimates) and β (which yielded sensitivity estimates). We evaluated the saturated model, in which prevalence and the sensitivity and specificity of the tests were estimated separately for each population, and several simplified models, in which test performance was assumed to be constant across populations.

Latent class models were fitted using non-linear weighted least squares (PROC NLIN, version 6.12 TS50; SAS Institute, Cary, NC). Standard errors were used to calculate 95% confidence intervals for parameter estimates; these intervals were truncated when they overlapped 0 or 1. Models were compared using standard likelihood ratio techniques based on the difference in their –2 log likelihoods.

RESULTS

HHV-8 seroprevalence and agreement among tests

A large proportion of African subjects were HHV-8-seropositive, based on any of the 3 serological assays (Table I). For example, with the K8.1 EIA, 82% of Congolese subjects, 87% of Botswana Sans and 76% of Botswana Bantus were seropositive. Nonetheless, the 3 tests gave different orderings of the African populations in terms of seroprevalence: the orf65 EIA ranked the Congolese highest in seroprevalence (79% seropositive), while the IFA and K8.1 EIA ranked the Sans highest (86% and 87%, respectively). For Maltese subjects, 2 tests gave relatively low seroprevalence estimates (16% by IFA, 9% by K8.1 EIA), whereas 1 test gave a substantially higher estimate (54% by orf65 EIA).

Overall, the level of beyond-chance agreement among assays ranged from slight to fair, with 3-way κ values ranging from 0.05 to 0.34 in the various populations (Table I). Pairwise agreement between the orf65 EIA and either of the other tests was especially poor (κ 0.03–0.24), except in the Congo population, where agreement between the orf65 and K8.1 EIAs was moderate (κ 0.43). Pairwise agreement between IFA and K8.1 EIA ranged from fair to moderate (κ 0.30–0.50).

Latent class models

Table II describes the fitted results of latent class models. Model 1 allowed each test's sensitivity and specificity to vary across the 4 populations. Using this model, estimates of the sensitivity and specificity of the K8.1 EIA were high and relatively constant across populations (91–100% and 92–100%, respectively). For the IFA, estimates of sensitivity were also reasonably high but somewhat variable (80–100%); specificity estimates for the IFA varied widely (57–100%). For the orf65 EIA, estimates of sensitivity and specificity were mostly lower than for the other 2 tests and displayed much more variability. Finally, prevalence of HHV-8 infection was estimated to be high in Africa (especially among Sans, 96% of whom were estimated to be infected) and lower in Malta (7%). In this model, estimates of test specificity in African populations were imprecise (reflected in wide confidence intervals, Table I), because few subjects were estimated to be uninfected. Similarly, estimates of test sensitivity in the Malta group were imprecise.

A difficulty with model 1 is that it is fully saturated; *i.e.*, for each population the number of parameters (7 parameters: π and each test's α and β) equals the number of independent cells in the $2 \times 2 \times 2$ table classifying the subjects by their test results. There is no way to check the fit of model 1 because the counts estimated by the model must equal the observed counts. However, the fit of simplified models, in which sensitivity and specificity of particular tests are held constant across populations, can be compared with the fit of model 1.

Results of model 1 suggested that the sensitivity and specificity of the K8.1 EIA were similar in the various populations (Table II). We therefore fitted model 2, in which the sensitivity and specificity of the K8.1 EIA were assumed to be constant across populations (Table II). The difference in –2 log likelihoods between models 2 and 1 was not significant (2.89 on 6 df, $p = 0.82$), indicating that model 2 provides an acceptable fit to the data. Under model 2, the K8.1 EIA had high sensitivity (100%) and specificity (98%) in all populations, and these parameters were precisely estimated.

Models 1 and 2 did not differ markedly in estimates of the sensitivity and specificity of the IFA and orf65 EIA or in estimates of HHV-8 prevalence in the various populations. Compared with model 1, model 2 estimated lower IFA specificity (40%) and slightly lower HHV-8 prevalence (87%) in the San population. These changes were related, in that model 2 characterized more of the IFA-seropositive Sans as false-positive than did model 1. Nonetheless, the importance of this difference is unclear, given the marked imprecision with which both models estimated IFA specificity in this population (Table II).

We examined further-simplified models in which the sensitivity and specificity of the IFA or of both the IFA and orf65 EIA were held constant across populations. These models fitted the data significantly less well than did model 2 and were rejected in favor of model 2 (data not shown).

Under the final model (model 2), the mean sensitivity of the orf65 EIA across populations was 76% and the mean sensitivity of the IFA was 88%. These values were significantly lower than the estimated 100% sensitivity of the K8.1 EIA (*t*-test, $p < 0.0001$ and $p = 0.01$, respectively). Similarly, under model 2, the mean specificity values of the orf65 EIA (57%) and of the IFA (69%) across populations were lower than the 98% specificity of the K8.1 EIA (*t*-test, $p < 0.0001$ and $p < 0.0001$, respectively).

DISCUSSION

The excellent sensitivity [100%, 95% confidence interval (CI) 96–100%] and specificity (98%, 94–100%) of the K8.1 EIA and the relative ease with which large numbers of specimens can be tested utilizing an EIA format commend this test for application to large-scale epidemiological studies. We found no evidence that its accuracy varied across the African and Maltese populations that we studied. Nonetheless, our results should be considered with caution because they may not be readily extrapolated to other assay formats or populations.

For example, in a prior study (Engels *et al.,* 2000), we applied the K8.1 EIA to samples from hemophilia patients and their spouses living in the United States, considering all members of this low-risk group to be HHV-8–uninfected. We obtained an estimate of specificity of 98% (since 2% of these persons were seropositive), which was identical to that of the present study. However, among subjects with Kaposi's sarcoma in our earlier study (Engels *et al.,* 2000), only 78% (95% CI 69–87) were seropositive using the K8.1 EIA, which led to a lower estimate of sensitivity. Similarly, Spira *et al.* (2000), using their own highly specific K8.1 EIA, found only 87% of persons with Kaposi's sarcoma to be seropositive. The notably higher sensitivity in the present work may be due to differences in our assay, which was slightly modified from the previous version (see Material and Methods). Alternatively, the higher sensitivity may be due to differences in study subjects. While most Kaposi's sarcoma patients have high antibody levels, a minority do not make detectable antibodies (Engels *et al.,* 2000;

TABLE I – HHV-8 SEROPREVALENCE ESTIMATES AND AGREEMENT AMONG TESTS

Population	Number	Seroprevalence, % (95% CI)			Agreement beyond chance, κ			
		Orf65 EIA	IFA	K8.1 EIA	3-way	Orf65 *vs.* IFA	Orf65 *vs.* K8.1	IFA *vs.* K8.1
Congo	321	79 (74–83)	69 (63–75)	82 (78–87)	0.34	0.17	0.43	0.46
Botswana San	155	61 (53–68)	86 (81–92)	87 (81–93)	0.06	0.05	0.03	0.30
Botswana Bantu	161	49 (41–57)	80 (73–86)	76 (69–84)	0.20	0.13	0.24	0.37
Malta	200	54 (47–61)	16 (11–21)	9 (5–13)	0.05	0.03	0.08	0.50

TABLE II – ESTIMATES OF TEST PERFORMANCE AND HHV-8 PREVALENCE DERIVED FROM LATENT CLASS MODELS

Parameter estimate	Model 1	Model 2
Orf65 EIA, sensitivity/specificity[1]		
Congo	87 (82–92)/60 (44–75)	87 (83–91)/59 (44–75)
Botswana San	62 (53–71)/67 (26–100)	62 (53–70)/45 (23–67)
Botswana Bantu	58 (47–69)/85 (65–100)	57 (48–66)/76 (62–90)
Malta	87 (68–100)/48 (41–56)	87 (68–100)/48 (41–56)
IFA, sensitivity/specificity[1]		
Congo	80 (74–86)/81 (66–96)	80 (75–85)/81 (66–95)
Botswana San	90 (81–100)/100 (0–100)	90 (86–95)/40 (17–63)
Botswana Bantu	89 (80–98)/57 (31–83)	88 (82–94)/47 (31–64)
Malta	100 (58–100)/90 (83–98)	99 (60–100)/90 (86–94)
K8.1 EIA, sensitivity/specificity[1]		
Congo	100 (96–100)/100 (73–100)	100 (96–100)/98 (94–100)
Botswana San	91 (82–100)/97 (0–100)	100 (96–100)/98 (94–100)
Botswana Bantu	94 (83–100)/92 (41–100)	100 (96–100)/98 (94–100)
Malta	100 (8–100)/98 (94–100)	100 (96–100)/98 (94–100)
Prevalence of HHV-8 infection[1]		
Congo	82 (75–89)	82 (77–87)
Botswana San	96 (82–100)	87 (80–93)
Botswana Bantu	80 (63–96)	76 (68–83)
Malta	7 (0–15)	7 (2–12)
Model parameters held constant across populations	None (saturated model)	K8.1 sensitivity and specificity
Model −2 log likelihood, df	2,399.93, 28	2,402.82, 22

[1]Values are percentages (95% CI).

Gao *et al.,* 1996; Simpson *et al.,* 1996), so relying on patients with Kaposi's sarcoma might previously have led us and others to under-estimate test sensitivity for detecting asymptomatic infection. Further work is needed to characterize the sensitivity of our K8.1 EIA in additional populations.

In the present study, the IFA, which is widely used in epidemiological research, appeared to be more sensitive and specific on average than the orf65 EIA, which has received more limited application, usually as part of a combination of assays (Simpson *et al.,* 1996; Renwick *et al.,* 1998; Melbye *et al.,* 1998). Nonetheless, both the IFA and orf65 EIA performed significantly worse than the K8.1 EIA. The accuracy of the IFA and orf65 EIA varied substantially across populations in our study. Both assays also had lower specificity in the African populations than in the U.S. subjects that we tested previously (Engels *et al.,* 2000): in our earlier work, we estimated specificity of 98% for the orf65 EIA and 88% for the IFA (having found that 2% and 12%, respectively, of U.S. hemophiliacs and their spouses were seropositive). Assays may perform poorly in the presence of generally high antibody levels, which are sometimes seen in African persons. This situation may create special difficulties for the IFA, where high antibody levels can make the identification of the diagnostic fluorescence pattern somewhat subjective. One also may speculate whether infection with another agent, related to HHV-8, could in part be responsible for the high false-positive rates sometimes seen (*e.g.,* 52% for the orf65 EIA in Malta).

Variable assay performance is problematic for epidemiological research, which depends on the reliable demonstration of differences across groups of interest. It is relatively straightforward to estimate infection prevalence, using a test with imperfect sensitivity and specificity, by taking appropriate account of test misclassification (Rogan and Gladen, 1978; Gastwirth, 1987). However, this correction requires knowledge of the test's sensitivity and specificity in the population being tested. Our finding that IFA and orf65 EIA performance varies across populations implies that interpretation of these tests' results would be difficult.

HHV-8 prevalence was very high (76–87%; Table 2, model 2) among African subjects, who were mostly young and middle-aged adults. These model-based prevalence estimates, implicitly adjusted for the imperfect sensitivity and specificity of the serological tests, generally confirm previous seroprevalence estimates for central and southern Africa (Olsen *et al.,* 1998; He *et al.,* 1998; Mayama *et al.,* 1998; Sitas *et al.,* 1999), which were highly dependent on the accuracy of particular tests. Our prevalence estimate for Congolese subjects is consistent with the extraordinarily high rates of Kaposi's sarcoma in this region, even in the absence of HIV infection (cumulative incidence 9 per 1,000 by age 64 years) (Cook-Mozaffari *et al.,* 1998). However, Kaposi's sarcoma incidence is not high in Botswana (Cook-Mozaffari *et al.,* 1998; Macrae and Cook, 1975), suggesting either that a necessary co-factor for Kaposi's sarcoma is lacking there or that the tumor is greatly under-reported.

HHV-8 prevalence was especially high among Sans, a comparatively small indigenous group who, at the time blood samples were obtained, were culturally isolated from neighboring Bantus. Their high prevalence implies that HHV-8 can adapt to spread efficiently in a genetically homogeneous host population. We previously observed similarly high HHV-8 prevalence among isolated Amerindian tribes in Central America (Biggar *et al.,* 2000).

By contrast, HHV-8 prevalence was much lower (7%) in elderly adults from Malta. Kaposi's sarcoma incidence in Malta, for adults 50 years of age or older, is 2.2 per 100,000 person-years for men and 1.8 per 100,000 person-years for women (Vitale *et al.,* in press). These rates, although several-fold higher than those reported for non-Mediterranean Europe or the United States (Hjalgrim *et al.,* 1996; Grulich *et al.,* 1992; Biggar *et al.,* 1984), are still much lower than those estimated for younger persons in Congo and other parts of sub-Saharan Africa (Cook-Mozaffari *et al.,* 1998; Oettle, 1962).

Our analytic models depended on a conditional independence assumption, which considered the errors of the various tests to be uncorrelated, conditional on individuals' true infection status. This assumption enabled us to express the probability of obtaining each particular combination of test results as the product of terms related to the error rates of the separate tests. The lack of agreement among HHV-8 tests that we observed (Table I), also reported by Rabkin et al. (1998), is generally consistent with this assumption since disagreements among tests arise when their errors (false-negative and false-positive) are not completely correlated. Nonetheless, there are several reasons why this assumption might not be valid here. First, all serological tests might miss the same fraction of truly infected individuals, leading to positive correlations among the false-negative errors. This concordance could occur, *e.g.,* if there is a subgroup of particularly immunocompromised infected persons who cannot make any HHV-8 antibodies. Second, all tests might be falsely positive for some individuals (leading to positive correlations among false-positive errors) if those subjects have generally high, non-specific antibody reactivity. Third, as is the case for Epstein-Barr virus (IARC, 1997), infected persons may make different antibodies at different stages of infection, which could lead to negative correlations among false-negative errors.

In analyses not shown here, we studied the effects of these potential violations of the conditional independence assumption on our parameter estimates. Following previous work by Vacek (1985) and Sinclair and Gastwirth (1996), we evaluated a relatively simple latent class model (2 populations, Congo and Malta; 2 tests, K8.1 EIA and IFA), in which we incorporated a range of possible correlations between test errors. In these analyses, prevalence estimates were 81–92% for Congo and 1–11% for Malta. Sensitivity and specificity of the K8.1 EIA ranged from 89% to 100% and from 92% to 100%, respectively, and the K8.1 EIA consistently had higher sensitivity and specificity than the IFA. Although these values for sensitivity and specificity of the K8.1 assay provide evidence of its high accuracy, the lower limits of these ranges imply that the assay might misidentify infection in some settings. Indeed, combinations of tests may perform better than any single test (Engels *et al.*, 2000), but we could not evaluate this possibility in our study.

In conclusion, we found HHV-8 prevalence to be high in each African population and lower in elderly adults from Malta. One test that we evaluated, the K8.1 EIA, demonstrated very high sensitivity and specificity. Two other tests, the IFA and the orf65 EIA, performed less well overall and had sensitivity and specificity that varied across populations. Studies in additional populations, using latent class models or other analytic approaches, are needed to validate our findings regarding the high accuracy of the K8.1 EIA and its role in conjunction with other serological assays for the diagnosis of HHV-8 infection.

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